PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
G01N 33/543, 33/558, B01L 3/00

A1

(11) International Publication Number: **WO 97/06437**

(43) International Publication Date: 20 February 1997 (20.02.97)

(21) International Application Number: PCT/EP96/03380

(22) International Filing Date: 30 July 1996 (30.07.96)

(30) Priority Data:
95202118.6 3 August 1995 (03.08.95) EP
(34) Countries for which the regional or international application was filed: NL et al.

(71) Applicant *(for all designated States except US)*: AKZO NOBEL N.V. [NL/NL]; Velperweg 76, NL-6824 BM Arnhem (NL).

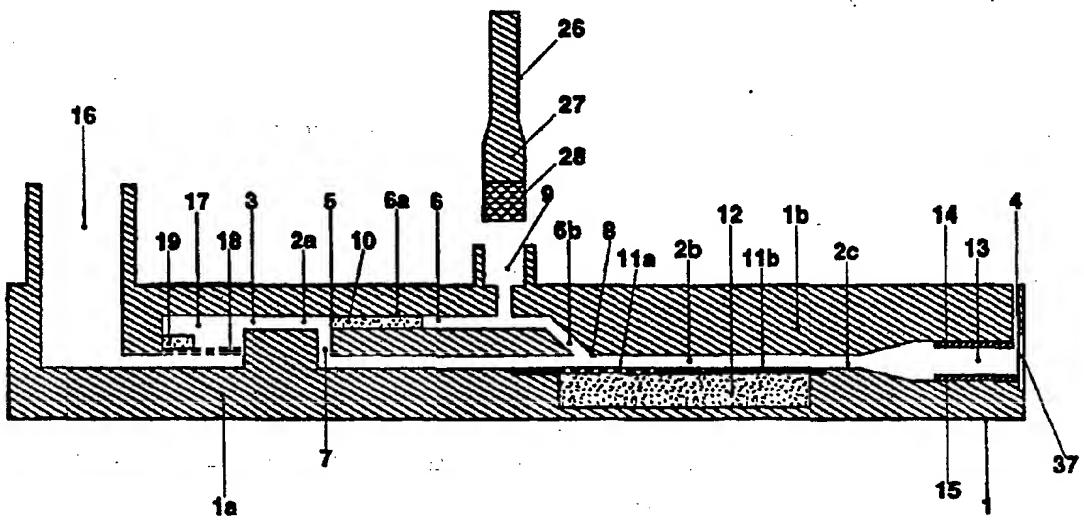
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VAN DAMME, Hendrik, Sibolt [NL/NL]; Bethaniestraat 9, NL-5211 LG Den Bosch (NL). PELSSERS, Eduard, Gerard, Marie [NL/NL]; Nistelrodestraat 55, NL-5045 MD Tilburg (NL). KAELEN, Henricus, Joseph, Ida, Theodorus [NL/NL]; Runrotstraat 13, NL-5386 CT Geffen (NL).

(74) Agent: HERMANS, F., G., M.; P.O. Box 20, NL-5340 BH Oss (NL).

(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DIAGNOSTIC DEVICE



(57) Abstract

The present invention describes a device for the detection or determination of the amount of an analyte in a test liquid in an autonomous way. The device comprises a transparent body provided with a conduit having an inlet and an outlet end. This conduit comprises a first junction branching off in a first branch and a second branch, and a second junction at which the first branch and second branch join. The first branch is provided with a sample inlet comprising assay reagents. The first branch and second branch are arranged such that, in use, a transporting liquid entering the inlet of the conduit is divided in said first banch and said second branch and promulgates a sample in the first branch through the second junction before the transporting liquid arrives at the second junction via the second branch. The outlet end of the conduit is provided with means for detection of the analyte. Optionally the conduit comprises a semi-permeable membrane between the second junction and the detection means to enable a bound/free separation step.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

Diagnostic device

The invention relates to a device for performing an assay for the detection or determination of the amount of an analyte in a test liquid.

Numerous assays have been developed for qualitative and quantitative detection of a variety of analytes such as those concerning the biochemical diagnosis of various human and animal disorders. There is a continuing interest to make these assays easier to perform and thus more available to non-technical personnel in a wide variety of environments, such as the doctors office, clinics, the home, crisis centres, emergency rooms, ambulances, blood banks, hospital laboratories etc. Current diagnostic instruments are especially intended for large scale testing and are in general rather complex to allow control over sample size, timing of addition of reagents and incubation time.

Such expensive instruments are not suited for small scale testing and in a non-laboratory environment. In these cases testing is mostly performed by manual procedures. Current manual procedures are often too delicate, requiring much skill, and are consequently not easy to perform by laymen or non skilled people. Manual tests especially designed for use by laymen, such as the so-called dipstick tests, exist but these are only suited for qualitative measurements.

The closest state of the art comprises a disposable diagnostic device and method of use described in WO 94/19484 (Biocircuits Corporation). This patent application relates to a device comprising a first and second flow path orthogonal to each other. The first flow path connects a sample port via a transport channel and an incubation area to a waste reservoir. The transport channel and/or incubation area comprises a reagent. The incubation area comprises a signal producing system and is underneath an optically clear window. The second flow path connects an inlet port via a side reagent reservoir and the incubation area to a side waste reservoir. The side reagent reservoir comprises for example a substrate if the signal producing system is enzyme based.

In use an analyte containing sample is introduced into the sample port and drawn by capillary action through the

transport channel into the incubation area. The analyte participates in the signal producing system in the incubation area. After a certain period of time a buffered wash solution is introduced via the sample port, displacing the sample to the waste reservoir. Then liquid is introduced into the inlet port at a certain point of time, which liquid dissolves the substrate and enters the incubation area where the visualisation reaction takes place. After a predetermined time one or more readings have to be made.

The device may comprise a capillary valve for enhanced control over liquid flow through the incubation area. In this way it is prevented that wash solution introduced via the sample port enters the side reagent reservoir or the side waste reservoir. A capillary valve may also be used to control the flow into and/or out of the incubation area.

From the above it is clear that, despite a significant improvement over the prior art, the device according to WO 94/19484 has several drawbacks. It is not able to perform an immunoassay procedure autonomously. That is, a plurality of manual operations are required, said operations being interrupted by waiting periods. To obviate this problem the patent application again relies on an apparatus. In addition, wash solution is introduced through the same flow path as the sample and thus the wash solution will be contaminated by analyte and reagent due to mixing and desorption of analyte and reagent from the walls of the transport channel, resulting in unsatisfactory washing at the incubation area. In addition, readings have to be performed after a specific predetermined period of time.

The object of the present invention is to provide a device capable of performing an assay, and in particular an immunoassay, requiring less manual operations with a reduced number of waiting periods.

This is achieved with a device for performing an assay for the detection or determination of the amount of an analyte in a liquid sample, characterized in that the device comprises a body provided with a conduit having an inlet end and an outlet end, said conduit comprising a first junction branching off into a first branch and a second branch, and a second junction at which said first branch and second branch join, wherein the first branch comprises a sample inlet, and the first

branch and second branch are arranged such that, in use, a transporting liquid entering the inlet of the conduit is divided in said first branch and said second branch and promulgates a sample in the first branch through the second junction before the transporting liquid arrives at the second junction via the second branch, and the outlet end being provided with detection means enabling the detection or determination of the amount of the analyte in the sample.

Thus, merely introducing liquid, such as washing or transporting liquid, through the inlet results in transport of the sample via the second junction into the conduit, while in addition to this the liquid arriving at the second junction via the second branch remains uncontaminated by analyte and/or reagent. The timing at which the liquid arrives at the second junction depends on the design chosen, not on the person performing the assay, which eliminates human errors.

Preferably the first branch is provided with an absorber element between the first junction and the sample inlet.

In use, liquid entering the first branch is absorbed quickly by the absorber element, slowing down or stopping the flow of liquid through the second branch. Air is expelled by the absorber pad, promulgating the sample through the first branch, via the second junction towards the outlet end before the liquid via the second branch reaches the second junction. Thus, the sample can be promulgated without contact, and thus dilution, with liquid from the first junction. After the absorber pad is saturated liquid is no longer conducted through the first branch.

In a preferred embodiment the device according to the invention is characterized in that the sample inlet comprises an outwardly projecting canal allowing the uptake of sample by capillary action into the first branch.

Thus the use of a pipette is obviated. The distal end of the outwardly projecting canal is simply immersed in the liquid sample and capillary forces introduce sample in the device.

Preferably the device is arranged to allow the uptake of a predetermined defin d volume of sample, which can for example be achieved using capillary valves, and which will be

explained in detail later. This embodiment obviates the use of a pipette or other sampling device requiring training to be used properly.

In a different embodiment the sample inlet is arranged to receive and hold an application rod comprising means for the uptake and release of sample liquid, a defined volume of sample liquid being released into the first branch when the application rod is received by the sample inlet.

This allows for the introduction of a defined volume of sample in the device using an application rod which can be more easily handled, for example for the uptake of sample liquid from a test tube.

Though of particular advantage with the present invention, it should be clear that an application rod may be used for the delivery of a defined sample volume to devices according to the state of the art.

According to a preferred embodiment the inlet end of the conduit comprises a container for transporting liquid.

Thus the use of a pump is obviated, further reducing the operational cost.

To further reduce the number of waiting periods, and allow autonomous operation of the device, a preferred embodiment of the invention is characterized in that said conduit comprises a membrane between said container and the first junction.

Introducing washing liquid in the container, prior to introduction of sample, results in a precisely timed assay. The membrane offers a means to control the flow rate of washing liquid and thus the time it takes for the washing liquid to reach the second junction. Thus, instead of having to add the sample after a particular period after having introduced the washing liquid, it can be added immediately after having introduced the washing liquid and no further attention is needed. The advantage of using a membrane is that, in contrast to for example a constriction in the conduit, it is not easily clogged by some dirt, such as a grain of sand or textile fibre.

According to a preferred embodiment of the invention, part of the wall of the conduit located between the second junction and the outlet end is formed by a semi-permeable membrane, and an absorber element is arranged immediately adjacent to said semi-permeable membrane outside of the conduit.

Thus after having arrived at the second junction first, the sample will contact the semi-permeable membrane and only large complexes, comprising analyte bound by the reagent, will be retained while free unreacted reagent and analyte will pass the membrane and be absorbed by the absorber element, together with the sample liquid. Thus a bound/free separation is accomplished. When the washing liquid having followed the second branch reaches the semi-permeable membrane, it can wash the large complexes effectively, especially since it is not contaminated by analyte or reagent from the first branch. After saturation of the absorber element, the large complexes are transported by the washing liquid towards the detection means.

Exemplary embodiments of the invention will now be described in detail with reference to the drawings in which:

Figure 1 is a schematic cross-sectional view of a first embodiment of the device according to the present invention;

Figure 2 shows a cross-section of a part comprising a junction in one embodiment of the invention;

Figure 3 shows a cross-section of a modification of the outlet end of the device;

Figures 4a and 4b show a cross-section of the sample inlet of the device;

Figure 5 shows a cross-section of a device according to the invention with multiple branches;

Figure 6 shows a cross-section of an alternative embodiment of a device according to the invention;

Figure 7 shows a cross-section of a bound/free separation part of the device.

Figures 8-10 show different embodiments of detection means applicable according to the present invention;

In Figure 1 a device 1 for performing assays, in particular immunoassays, is shown comprising a conduit 2 with an inlet end 3 and an outlet end 4. The conduit 2 comprises a first junction 5 branching of into a first branch 6 and a second branch 7. The first branch 6 and the second branch 7 join at second junction 8. The first branch 6 comprises a sample inlet 9.

In use washing liquid is introduced via the inlet end 3 into the conduit 2. Subsequently an analyte containing sample

is introduced into the sample inlet 9. Said washing liquid is transported by capillary action, hydrostatic pressure, gravitational force, a simple pump or otherwise from the inlet end 3 to the outlet end 4. As it is generally preferred to perform assays on small samples, the channels will be small for which reason capillary action will usually be the essential force for transport of liquid. When the washing liquid reaches the first junction 5, it is divided over the two branches 6 and 7. The sample in branch 6 is promulgated via branch 6b and the second junction 8 towards the outlet end 4. To this end, the branch 6a is preferably provided with means that promulgates the sample mediated by gas in the branch 6 in the conduit 2b. In the embodiment shown in figure 1 this means comprises an absorber element 10. Washing liquid reaching the first junction 5 will be absorbed by the absorber element 10. Gas contained in the absorber element 10 is expelled in the process and this gas forces the sample in branch 6 via the second junction 8 into the conduit 2b. As soon as the absorber element 10 is saturated, the flow of washing liquid through branch 6 stops. This prevents washing liquid from passing via branch 6 to the conduit 2b and thus avoids contamination of the washing liquid with the analyte or a reagent present. The absorber element 10 is of porous material such as fibre material like cellulose, nylon, glass, cotton, polyester or acrylic fibres, ceramics or any other material expelling gas when wetted by the liquid.

For the detection of the analyte the device 1 may be provided with one or more reagents. The reagent may be added together with the sample, but it is preferred that the sample inlet 9 comprises the reagent. Thus the reaction starts when the sample is introduced in the device 1. If the reaction requires more than one reagent these can be included in the sample, the sample inlet or both, as desired.

In an alternative embodiment the reagent is contained in the branch 6b. Thus, the reaction is started only when the sample is promulgat d in branch 6b towards the second junction 8. This allows for very precise control over the onset of the reaction, independent of when washing liquid is introduced into the inlet end 3. This embodiment is particularly advantageous for fast reactions.

These reagents may comprise various components required to perform an assay for detection of an analyte in a test liquid, such as antigens or fragments thereof, antibodies or fragments thereof, DNA or RNA or fragments thereof, other members of specific binding pairs atc. These components may be in an unlabeled and/or labeled form. Suitable labels include enzymes, fluorescent compounds, chemiluminescent compounds, particulate labels such as gold sols and dyestuff sols. Furthermore these components may be coupled to a dispersed solid phase material to enable an adequate separation between the fraction bound to, for example, an antibody, and the unbound (free) fraction. Suitable solid phase materials are for example polystyrene latices.

The embodiment shown in figure 1 is provided with means for the separation of large complexes formed between reagent and analyte on the one hand and unreacted analyte and reagent on the other hand. The means comprise a semi-permeable membrane 11 forming a part of the wall of the conduit 2b and an absorber element 12 immediately adjacent to said semi-permeable membrane 11 outside the conduit 2b. In this application the means will be referred to as a bound/free separator and is described in detail in our pending European application EP 480 497, the description of which is herein incorporated by reference.

The semi-permeable membrane 11 is preferably an absolute membrane. This type of membranes is characterized by the fact that liquid flow through the membrane occurs only perpendicular to the membrane surface. In addition these absolute membranes do no contain so-called dead spaces, as for instance in tortuous membranes, which can hold a certain amount of the reaction mixture withstanding the suction action of the absorber element. Reagent from from these dead spaces may diffuse back into the conduit 2b, thereby decreasing the efficiency of the separation process.

In order to prevent non-specific binding of various components to the membrane 11, this membrane 11 can be coated for example with a tri-block copolymer of polyethylene oxide and polypropylene oxide, such as F108 Synperonic (ICI Surfactants). In this way especially a solid phase dispersion, such as a dispersion of polystyrene latex or gold particles which are very

suitable for several types of immunoassay that can be performed using the device according to the invention, can be prevented from attaching to the membrane.

Examples of absolute membranes are track etched membranes, such as cyclopore membranes (Whatman, Belgium) and Nucleopore membranes (Nucleopore, USA). Another example of absolute membranes are membranes produced by combined lithographic and etch techniques such as microsieves (Aquamarijn Microfiltration, the Netherlands).

In use the analyte present in the sample will react with the reagents, for example a labeled component and a component coupled to a dispersed solid phase, forming large complexes. The device 1 according to the invention allows control over the time allowed for the reaction to proceed, as will be discussed shortly, and the reagent containing sample will not arrive at the bound/free separator before the reaction is complete. At the bound/free separator all the sample liquid together with unreacted reagent and analyte, if any, passes through the semi-permeable membrane 11 and is absorbed by the absorber element 12. The large complexes however are retained by the semi-permeable membrane 11. The device 1 is arranged in such a way that the washing liquid, coming from the second branch 7, arrives at the semi-permeable membrane 11 only after the complete absorption of the sample liquid. The capacity of the absorber element 12 to absorb liquid is larger than the volume of the sample liquid. Thus, when the washing liquid reaches the semi-permeable membrane 11, it is absorbed by the absorber element 12, resulting in a very effective washing of the retained large complexes and the substantially complete removal of unreacted reagent. It is advantageous if the large complexes are retained in a very small area of the semi-permeable membrane only, the area having reference number 11a. Thus all liquid passes this small area which results in a very effective washing.

To minimise contamination of washing liquid with reagent, the semi-permeable membrane 11 is located at or near the second junction 8, as a result of which the walls of conduit 2b will be contacted with and contaminated with free reagent as little as possible. To prevent the sample liquid/reagent from entering the second branch 7 a pressure barrier, for example an

abrupt increase in diameter, increase in hydrophibicity etc., may be provided.

To restrict the area of the semi-permeable membrane 11 serving as a wash area, the conduit 2b can be provided with a moderate pressure barrier. To increase the absorbance capacity, the conduit 2b may comprise two absorber elements 12 opposite to each other, or the conduit may over a part of its length be surrounded by the absorber element 12.

To increase the capacity of the absorber element 12 it is mounted over a long distance along the conduit 2b. It is remarked that especially the first liquid entering the absorber element 12 will contain reagent. This reagent will proceed towards the distal end of the device 1. There is no flow of liquid in the lumen of the conduit 2b until the absorber element 12 is saturated. When the the absorber element 12 is saturated, a high concentration of reagent is present at the distal end of the absorber element 12. If the membrane 11 were permeable at 11b, reagent could diffuse back into the carefully washed large complexes containing liquid. This would to a certain extent negate the effect of washing. Therefore the part 11b of the semi-permeable membrane may be permeable for gas contained in the absorber element 12 only. Alternatively, the semi-permeable membrane 11 may be made impervious over part of its surface, for example by treating it with chemicals that make the membrane impervious, by covering it, for example with adhesive tape, etc. or a semi-permeable membrane 11 can be used which during manufacture was provided with pores in only part 11a of it. Other possibilities to prevent reagent from re-entering the liquid are possible.In the bound/free separator shown in figure 7 the conduit 2a, the first branch 6 and the second branch 7 are diverting from the surface of the semi-permeable membrane 11a. Therefore the use of an impervious part of the membrane is not necessary. Another possibility is the use of an absorber element 12 which not only absorbes liquid but also binds reagent, for example by ionic int raction.

The semi-permeable membrane 11 and the absorber element 12 should contact each other over preferably the full surface of the semi-permeable membrane 11. This may be achieved using physical techniques such as pressure, or by lasting physi-

cal contact, for example by heat-sealing membrane 11 and absorber element 12 together.

It should be clear that the features described above for the bound/free separator can also be used in combination with the bound/free separation device described in our pending European application EP 480 497.

When the absorber element 12 is saturated, the large complexes are transported by the washing liquid to a detection means, in the embodiment shown in figure 1 a detection chamber 13. This detection chamber 13 may contain further reagents 14 and 15, for example enzyme substrate and chromogen. In the embodiment shown the detection chamber 13 is provided with a transparent window 37, allowing visual inspection or spectrophometric reading such as absorption measurement, fluorescence measurement, as well as nephelometric measurement etc.

Said reagents 14, 15 may also be provided in the conduit 2c. or alternatively in a third branch (not shown), branching of from conduit 2a or branch 7. These reagents are delivered automatically after the bound/free separation reaction is completed. It should be clear that one or more reagents may be provided at certain places or delivered at certain places as needed for a particular assay. Thus it is also possible to avoid compounds present in the sample for interfering with further reagents such as substrates, because the compounds are removed during the bound/free separation before the bound analyte is transported to the further reagent or the further reagent is delivered.

As described earlier, the sample is introduced into the device 1 immediately after the introduction of the washing liquid. The time available for reaction between analyte and reagent depends on the design of the device 1. If the operation of the device 1 essentially relies on capillary action, the device 1 is preferably provided with a container 16 at the inlet end 3, connected to a filler chamber 17, via a membrane 18. The liquid has to pass membrane 18 which provides flow resistance. Other ways of providing flow resistance are possible but the use of a membrane 18 is advantageous in that it is not easily blocked by air, dirt such as a grain of sand etc. Suitable membranes are those with transmembrane fluxes in the order of 1

to 200 ml/min/cm^2 at 10 PSI. Hydrophilic membranes are preferred as these are more easily wetted by the liquid. To improve the proper functioning the membrane 18 a tiny absorber element 19 in the filler chamber 17, immediately adjacent to and covering only part of the membrane 18, may be provided, facilitating the initial release of the liquid by the membrane 18. This absorber element is preferably made of glass fibre.

The reproducibility of the time delay depends on the uniformity of the trans-membrane flux of the type of membrane used. By placing the membrane in a horizontal position and feeding the liquid from below, air inclusion influencing the trans-membrane flux, can be prevented.

Though of particular advantage with the present invention, it is remarked that such a time delay element, comprising a container, membrane and filler chamber, can be used with devices according to the state of the art as well, to provide for the timed delivery of a washing liquid.

The device according to the invention can be considered as a combination of elements, each capable of performing one or more assay steps, alone or in combination, in an autonomous way. These elements are combined to carry out a complete assay and can be used in different combinations to carry out a different process. The device takes care of complex fluid movements and makes apparatus with complex mechanics and control software superfluous.

It is possible to manufacture the different elements as separate parts that can be used as building blocks to assemble a device according to the invention. Thus a device can be assembled useful for the desired assay. It is also possible that one of the elements, for example the sample inlet element contains different reagents for different types of assay.

As mentioned earlier, small sample sizes are generally preferred. This allows the device 1 to be operated using capillary forces, obviating costly pumping means. The shape of the conduit 2 may vary, but for capillary action to occur two opposite walls of the conduit 2 are preferably between 0.01 and 2 mm apart.

To prevent the liquid from l aving the device 1, the outlet end 4 should act as a pressure barrier, using a sudden increase in diameter, hydrophobic coating etc.

Further control over period of time for reaction can be achieved in several ways. For example, the conduit 2a and the second branch 7 can be or be made more or less hydrophobic, for example by chemical treatment, coating hydrophobic compounds with for example using Teflon spray, fluor-resin or vaseline, and other methods known per se, and/or the diameter and length of the conduit 2a can be varied. In addition the conduit 2a and second branch 7 may be provided with delay means 20, as shown in figure 5, comprising an absorber element 21 and a vent 22. Liquid from the container 16' is absorbed by the absorber element 21 and no liquid is passed on until the absorber element 21 is saturated with liquid. Gas contained by the absorber element 21 is discharged to the atmosphere via vent 22.

To avoid the risc of air getting trapped before absorber element 10, thus preventing (timely) absorption of liquid and consequently impeding transport of the sample into the conduit 2b, the absorber element 10 may be shaped as shown in figure 2. The absorber element extends over a part of the wall of conduit 2a. In the embodiment of the absorber element 10 shown, it has also been given a larger dimension over part of its length in branch 6, to increase the volume of gas the absorber element 10 can expell, necessary for the promulgation of the sample.

The detection means may overlap with the bound/free separator, as is shown in figure 3. A transparent device 1 allows visual inspection or quantitative measuments with a suitable apparatus.

As shown in figuur 4a, the sample inlet 9 may be provided with a filter 23 allowing removal of lumps, dirt or for example blood cells. Reagent may be provided below the filter (not shown). According to a preferred embodiment the sample inlet 9 can be provided with a reagent 24 containing holding means 25. Thus a device 1 can be provided with one of a multitude of holding means 25 each containing different reagents 24, which offers a choice of assays to be performed with the device 1. In the embodiment shown the holding means 25 also comprises a filter 23, allowing the removal of cells, such as red blood cells, dust etc.

According to a preferred embodiment sample liquid is introduced in the device 1 by means of an application rod 26

comprising a handle 27 provided with compressible porous material 28 at one end thereof. This porous material 28 is capable of taking up a defined volume of sample liquid which volume is governed by the amount and capacity of said porous material 28. Suitable compressible porous materials are for example Porex porous material (EDP x-2124), and preferably 3M foam type 1563. Optionally the compressible porous material 28 can be hydrophilized by plasma treatment or treatment with surfactants such as a mixture of poly(oxyethylene) sorbitane mono-laurate (Tween 20) and sorbitane-monooleate (Span 80).

The compressible porous material 28 is dipped into a sample liquid whereby a defined sample volume is taken up. Subsequently the application rod 26 is inserted in the sample inlet 9 and the porous material 28 is compressed, whereby this defined sample volume is released. To this end the handle 27 engages the sample inlet 9 in an airtight fashion, while a sudden decrease in cross-section of the sample inlet 9 provides a surface against which the porous material 28 is compressed. The released volume of sample should be such that it cannot come into contact with the absorber element 10 in the first branch 6a. In an alternative embodiment the compressible porous material 28 can be provided with a reagent, offering the same advantage as the holding means 25 described above.

The reagents for the device 1, holding means 25 and application rod 26 are usually provided in a lyophilized form, though other forms are possible, for example as a coating obtained by evaporation of a solvent. In a preferred embodiment the substrate and chromogen are added in insoluble, inert, porous pads. Suitable materials for the pads are for example Nylon membrane (MSI, USA) with a pore size of 0,2 tot 1,0 mm. Said pads do not block the flow of liquid and allow rapid release of reagent into the liquid.

Another embodiment of the device 1 according to the invention is shown in figure 5. The device 1 comprises a conduit 2, container 16, junctions 5, 8, branches 6, 7 and absorber element 10 corresponding to those described with reference to figure 1.

The sample inlet 9 comprises an outwardly projecting canal 29 allowing taking up sample liquid by capillary action by dipping the distal end of the canal 29 in the sample liquid. The

volume of sample liquid introduced in branch 6 is defined by means for stopping the flow of liquid by capillary action. Thus the branch 6a and 6b can each be provided with for example hydrophobic walls over part of the length of each branch 6a, 6b. It should be clear that such a sample inlet can also be used separately or in combination with another device for the uptake of a defined sample volume. In the embodiment shown in figure 5, an abrupt increase in diameter of the branches 6a and 6b provides a pressure barrier which can not be overcome, or be overcome quickly, by the sample liquid. However, the pressure built up by gas expelled by the absorber element 10, as discussed in relationship to figure 1, is sufficient to promulgate a defined volume of sample liquid into conduit 2b. A bound/free separator is provided in conduit 2b, comprising a semi-permeable membrane 11 and an absorber element 12. Washing liquid in a container 16', which may or may not be the same as container 16, is delayed by delay means 20, discussed earlier, and arrives at junction 30 with branches 31 and 32, which branches 31, 32 join at junction 33, only after the large complexes retained by the bound/free separator have been transported into the branch 31. The flow of liquid through conduit 2b stops automatically due to the pressure barriers provided in branch 31. Again an absorber element 34 is used to promulgate the liquid in the branch 31, containing the large complexes, into a conduit 35b to a second bound/free separator for a second washing step. From this example it is evident that the device according to the invention allows excellent control over the course of the reaction by appropriate design of the device. A third branch as described, or more additional branches, can be used to deliver reagents as needed for the particular assay. Thus the present invention allows a wide range of assays to be performed with a very limited operations to be performed by personell which do not require much training or skill. Miminal instructions suffice.

It is possible to provide a device having one container and serveral conduits 2', 2'' etc. each containing a sample, a blank and/or a standard solution. Thus adding liquid to the container 16 starts all assays at the same time, allowing for multiple, control and reference measurements

To exemplify the excellent fluid control provided by the present invention the working of the embodiment of a device

according to the invention shown in figure 6 is discussed in detail. Washing liquid from the inlet end 3 is divided at junction 5. Washing liquid absorbed by the absorber element 10 expels gas contained in the absorber element 10, promulgating an assay mixture, comprised of sample liquid introduced via sample inlet 9 and reagent, into conduit 2b to the semi-permeable membrane 11. Washing liquid passing through the second branch 7 is divided at a junction 41. Washing liquid passing into branch 42 dissolves substrate/chromogen reagents 14,15 present in this branch 42. The flow of liquid is stopped by the pressure barrier 44. Washing liquid passing from the junction 41 to the second junction 8 flows through conduit 2b and is absorbed by the absorber element 12, washing retained large complexes in the process. As soon as the liquid reaches the outlet end 4, gas can no longer be discharged to the surroundings. The absorber element 12 however is not yet saturated with liquid and liquid continues to be absorbed. The gas expelled from the absorber element 12 in the process results in an increased pressure in chamber 45, as a result of which the dissolved substrate is passed via junction 41 to the semi-permeable membrane 11 retaining the large complexes. There, an enzyme reaction may occur, depending on the presence of analyte in the sample. It is clear that the design of the device has to be well-considered. Once designed however, the personnel has no worries over its intricacy and can perform an assay with a mimimum of operations and no control apparatus.

From the above it should be clear that with a device according to the invention gas expelled by an absorber not only can be used to influence the flow of liquid downstream but also upstream.

Figure 8 shows a different embodiment of the detection means, the outlet end 104 being provided with a prism 36 which facilitates visual inspection and/or measurement of the detection reaction. The prism 36 may have a convex surface at the distal end, in effect forming a convex lens, to further facilitate inspection of the detection chamber 13. The conduit 2b is provided with reagents 14, 15 which are for example a substrate for an enzyme, and a chromogen. Here the conduit 2b doubles as the detection chamber 13.

The embodiment shown in figure 9 is similar to the one shown in figure 8, but here an adsorber 38 is provided that can be inspected, for example visually. An absorber element 39 is provided to facilitate the passage of liquid through the adsorber 38. It may also be advantageous to incorporate a reagent, such as one for stopping an enzyme reaction, in the adsorber 38. The adsorber 38 may concentrate any coloured product formed, increasing the sensitivity of the assay. Suitable adsorbers 38 depend on the particular dye used or produced, but Nylon membrane filters (MSI, USA) and preferrably GF/SE 30 paper (Whatman, UK) appear to be satisfactory.

Measurements with the device according to the invention is not limited to optical detection, in contrast, a host of detection methods is possible. As an example, figure 10 shows a detection chamber 13 provided with electrodes 40, 40' and reagents 14, 15, allowing electrochemical detection of reaction products. An enzyme suitable for electrochemical detection is glucose-oxidase (GOD) with D-glucose and potassium hexacyanoferrate as substrates.

The device 1 can be made of various materials, preferably of transparent plastics such as polystyrene, polycarbonate and polymethylmethacrylate.

Preferably the device comprises two parts 1a, 1b, of which for optical assays usually at least one will be of transparent plastic. If a window is provided for the detection chamber 13 the parts 1a, 1b may be opaque.

The parts 1a, 1b are advantageously formed by injection molding, and contain the conduit 2 and other elements as recesses. Advantageously the recesses all lie in one plane, but this is not required. For assembly, the parts are provided with aborber elements, membranes, reagents, hydrophobic material to act as pressure barriers etc. as needed for the intended assay. To assure satisfactory contact between a membrane and an absorber element, the absorber may advantageously be placed in a cavity of one of the parts, the depth of said cavity being slightly less than the thickness of the absorber element.

Using the device shown in figure 1, a sample can be introduced, said sample dissolving solid reagent comprising antibodies dir cted against the analyte and labeled with an

enzyme, and latex particles coated with antibodies directed against the analyte, said sample and dissolved reagent plus latex particles forming a assay mixture. Prior to the introduction of the sample, washing liquid is introduced in the container 16. From there the liquid passes the membrane 18 and enters the conduit 2a. At the first junction 5 the liquid is absorbed by the absorber element 10 which expels gas in the process. This gas promulgates the assay mixture via the second junction 8 to the semi-permeable membrane 11a, where the assay mixture is absorbed by the absorber element 12, in effect stopping the immunological reaction, which should be completed by then anyway, while the latex particles are retained. After the saturation of the absorber element 10, washing liquid passes through the second branch 7 via the second junction 8 to the semi-permeable membrane 11a. The retained latex particles coated with antibodies which have bound the analyte present in the sample to which in turn antibodies conjugated with enzyme have bound, are washed with the washing liquid until the absorber element 12 is saturated. Then the latex particles are transported by the washing liquid to the detection chamber 13, where substrate is provided for the enzyme. After a predetermined time the colouration of the solution is recorded, with the naked eye or using an apparatus. Thus, the only operations that have to be performed are: introduction of washing liquid, introduction of sample and, after a certain period of time, reading of the detection reaction.

Being cheap to manufacture the devices are intended for single use, though reuse is not excluded.

It will be clear that the invention allows many design variations of device according to the invention, allowing many different types of assay including several types of immunassay to be performed, using the timed flow control and improved washing provided by the present invention.

The present invention will now be further particularly described with reference to the following Example.
The device used is that depictured in Figure 1.

Example

1. Preparation of HIV-1 glycoprotein 160 coupled to polystyrene latex particles (solid phase coupled gp 160).

To 2 ml of aldehyde activated polystyrene latex particles (29.73 µmol aldehydes per m^2 latex, solid volume fraction = 2%) with a diameter of 723 nm, an amount of 0.4 ml phosphate buffer (500 mM, pH = 7) was added. Subsequently 1.2 ml of a HIV glycoprotein 160 (gp 160) solution (0.8 mg/ml) was added, followed by the addition of 0.4 ml of a $NaCNBH_3$ solution (250 mM). The reaction mixture was then incubated for 4 hours at ambient temperature. After incubation, the dispersion was centrifuged for 10 min. at 10 000xg and the pellet redispersed in a 50 mM glycine buffer, pH 8.5, containing 1 g/l BSA and 1 g/l SDS. The latex particles carrying the gp 160, were washed twice with this glycine/BSA/SDS buffer and subsequently three times with the same buffer but without SDS. Finally the pellet was redispersed in said glycine/BSA buffer and stored at 4°C.

2. Preparation of horse radish peroxidase (HRP) labelled gp 160 (gp 160 conjugate).

220 mg HRP was dissolved in 2.81 ml NaH_2PO_4 buffer (12 g/l), pH 7.5, containing 5.85 g/l NaCl, and incubated during 30 min. with 0.22 ml of a solution of SPDP in ethanol (12.5 g/l). The obtained HRP-SPDP was purified on a PD10 column and eluted with a NaH_2PO_4 buffer (12 g/l), pH 7.4, containing 5.85 g/l NaCl and 1.86 g/l dinatriumedetaat.$2H_2O$. To 12 ml of a solution of gp 160 (2 mg/ml), 0.12 ml of a DTT solution in water (154 g/l) was added and incubated for 30 min. at ambient temperature. After elution of the gp 160-DTT mixture over a G25 column, the gp 160-DTT was added to 3 ml HRP-SPDP whereupon the mixture was incubated for 120 min. at ambient temperature in the dark. This conjugate was frozen and stored below - 50°C.

3. Preparation of immobilized substrate reagents.

a. Preparation of TMB pads.

Tetramethylbenzidine hydrochloride (TMB) was dissolved in a solution of 9.1 mg citric acid/ml, 1 mg di-sodium EDTA/ml, 156.5 mg PEG-8000/ml in a concentration of 8 mg TMB/ml. 10 µl of this solution was sprayed per square cm MSI nylon membrane with a pore size of 0.22 µm. After spraying the pads were dried under nitrogen and subsequently cut into pieces of 10 by 1mm. These pads were then placed and fixed in the detection chamber (13) of the device (1) by slight mechanical pressure.

b. Preparation of sodiumperborate-tetrahydrate pads. Sodiumperborate-tetrahydrate was dissolved in a solution of 44 mg citric acid/ml, 80 mg sodium citrate, 2.6 mg di-sodium EDTA/ml and 156.5 mg PEG 8000/ml in a concentration of 20 mg/ml. 10 µl of this solution was sprayed per square meter nylon membrane with a pore size of 0.22 µm. The pads were further handled as decribed for the TMB pads.

4. Preparation of the device containing the assay reagents.

The device used is that depictured in Figure 1. The conduit (2a, 2b, 2c) and its branches (6, 7) have a cross-section of 2 mm by 0.1mm. The porous material (28) of the application rod (26) is capable to take up 30µl of liquid. The semi-permeable membrane (11a) used is a Cyclopore membrane (Whatman sa, Belgium), with a pore size of 0.6 µm.
Samples of 15µl containing 5% mannitol, 5% trehalose, 5% NSS, 0.81 g/l sodiumthiocyanate, 0.91 µg/ml gp 160-HRP conjugate and 0.0425% gp 160 coupled to latex, were applied to holding means (25) as depictured in Figure 4b. Subsequently these samples were freeze dried in these holding means, whereupon the holding means (25) containg the reagents (24), were placed in the sample inlet (9) of the device (1).

5. Assay method.

Distilled water is added to the container (16) of the device(1) in a volume of 5 ml, which is indicated by a mark on the wall of the container. Immediately after this handling the application rod (26) is inserted in the sample liquid, whereby 30 µl of

sample liquid is taken up. The application rod is then inserted in the sample inlet (9) and the liquid released by pressing. After 15 min. the colour is read through the window (37) and a slight to blue colour is an indication of a positive sample. The colour of a negative sample is not visually detectable. In this way an amount of 20 ng/ml of human monoclonal anti-gp 41 in serum can be detected.

Claims

1. Device for performing an assay for the detection or determination of the amount of an analyte in a liquid sample, characterized in that the device comprises a body provided with a conduit having an inlet end and an outlet end, said conduit comprising a first junction branching off in a first branch and a second branch, and a second junction at which said first branch and second branch join, wherein the first branch comprises a sample inlet, and the first branch and second branch are arranged such that, in use, a transporting liquid entering the inlet of the conduit is divided in said first branch and said second branch and promulgates a sample in the first branch through the second junction before the transporting liquid arrives at the second junction via the second branch, and the outlet end being provided with detection means enabling the detection or determination of the amount of the analyte in the sample.

2. Device according to claim 1, characterized in that the conduit is arranged to allow the transporting liquid to flow under capillary action.

3. Device according to claim 1 or 2, characterized in that the first branch is provided with an absorber pad between the first junction and the sample inlet.

4. Device according to anay of the preceding claims, characterized in that the sample inlet comprises an outwardly projecting canal allowing the uptake of sample by capillary action into the first branch.

5. Device according to claim 4, characterized in that the sample inlet and the first branch are arranged to allow the uptake of a defined volume of sample.

6. Device according to claim 4, characterized in that said sample inlet comprises assay reagents.

7. Device according to any of claims 1 to 3, characterized in that the sample inlet is arranged to receive and hold an application rod comprising means for the uptake and release of sample liquid, a defined volume of sample liquid being released into the first branch when the application rod is received by the sample inlet.

8. Device according to claims 1 to 3, characterized in that the sample inlet is arranged to receive and hold a filter holder, comprising a filter and assay reagents, whereby said filter holder is arranged to receive and hold an application rod, a defined volume of sample being released into the first branch when the application rod is received by the filter holder.

9. Device according to any of the preceding claims, characterized in that the inlet end of the conduit comprises a container for transporting liquid.

10. Device according to claim 9, characterized in that said conduit comprises a membrane between said container and the first junction.

11. Device according to any of the preceding claims, characterized in that part of the wall of the conduit located between the second junction and the outlet end is formed by a semi-permeable membrane, and an absorber element is arranged immediately adjacent to said semi-permeable membrane outside of the conduit.

12. Device according to any of the preceding claims, characterized in that said detection means comprises a conduit ending in a detection chamber.

13. Device according to claim 12, characterized in that the detection chamber is provided with reagents for detection of the analyte.

14. Device according to claim 12 or 13, characterized in that the wall of the detection chamber is provided with an adsorbing material and an absorber is arranged immediately adjacent to said adsorbing material outside of the detection chamber.

15. Device according to any of the preceding claims, characterized in that the device comprises a third branch to allow further control over the assay.

fig. 1

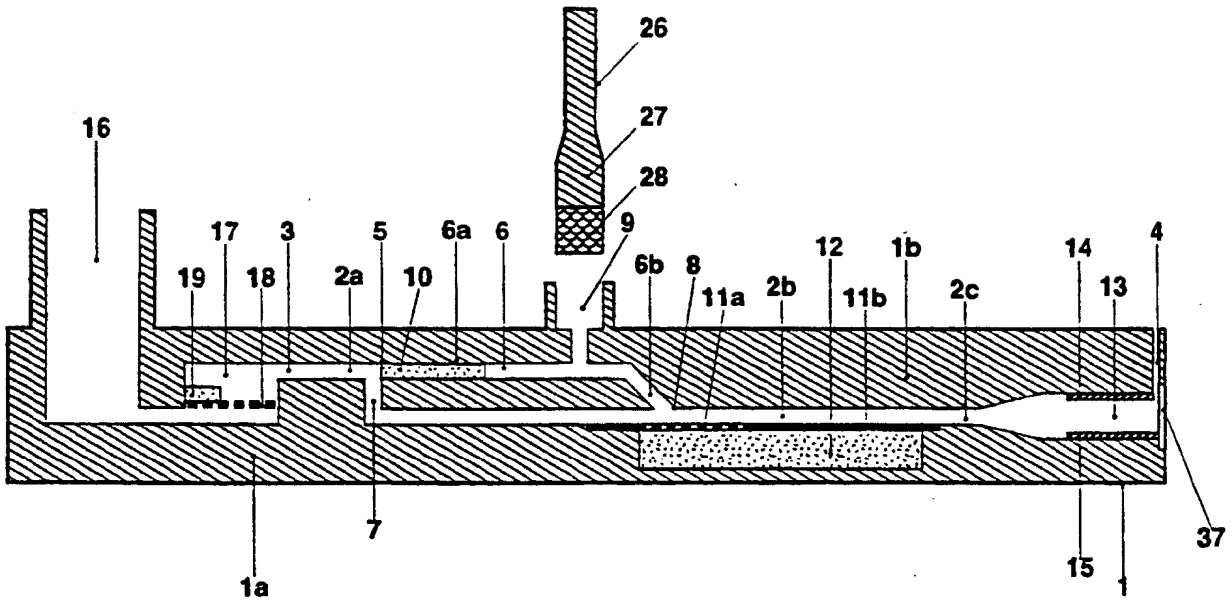

26
27
28
9
16
17
3
5
6a
6
19
18
2a
10
6b
8
11a
2b
12
11b
1b
2c
14
13
4
7
1a
15
1
37

fig. 2

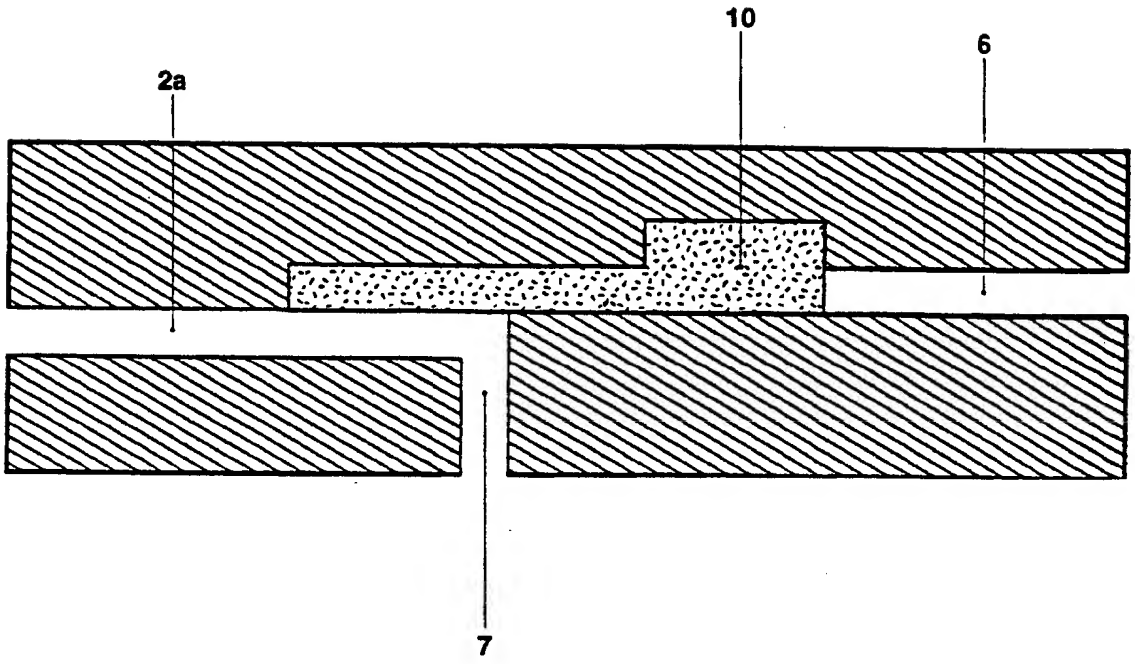


fig. 3

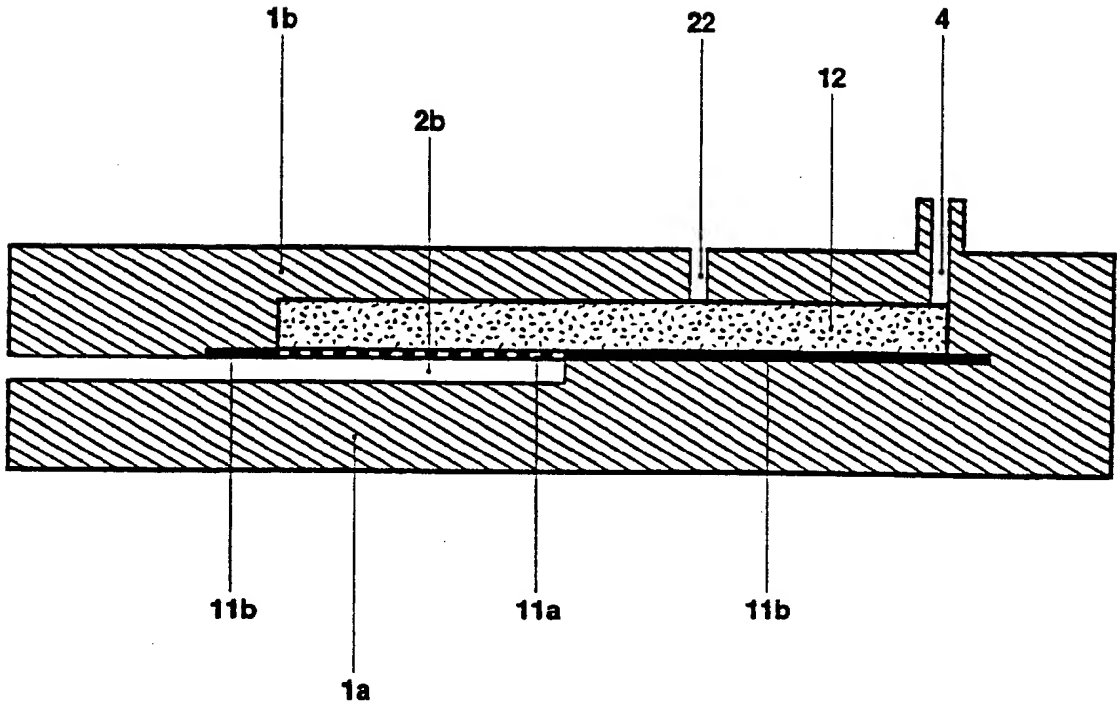


fig. 4

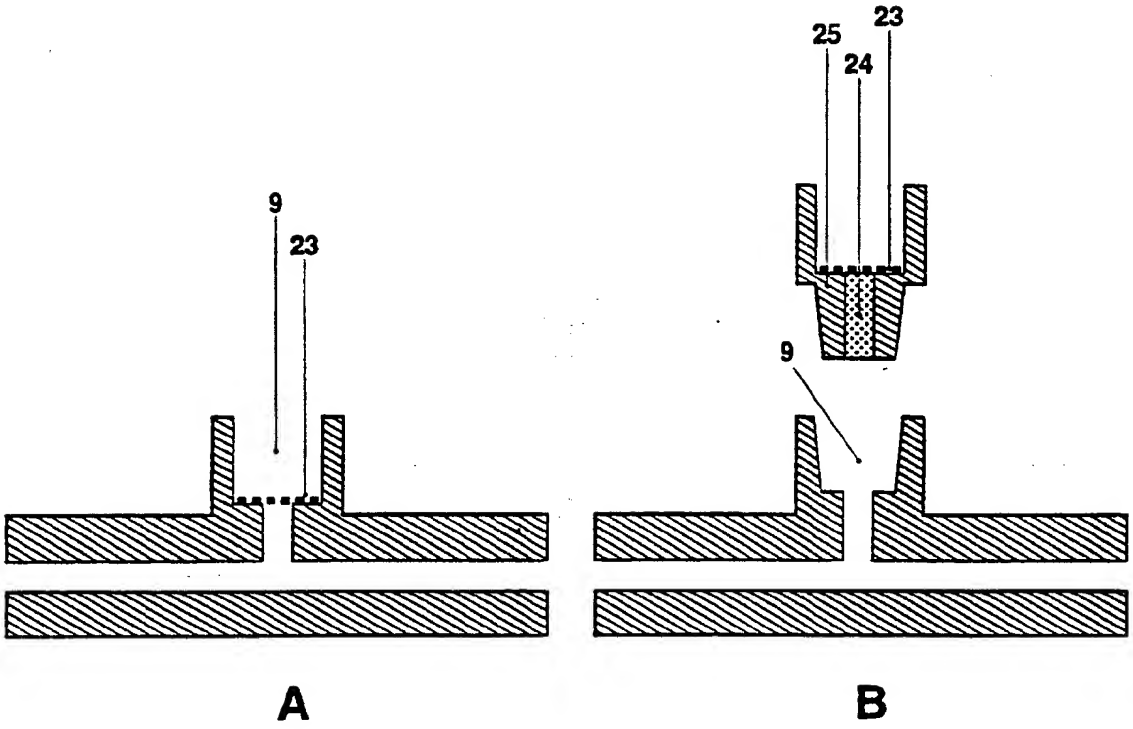


fig. 5

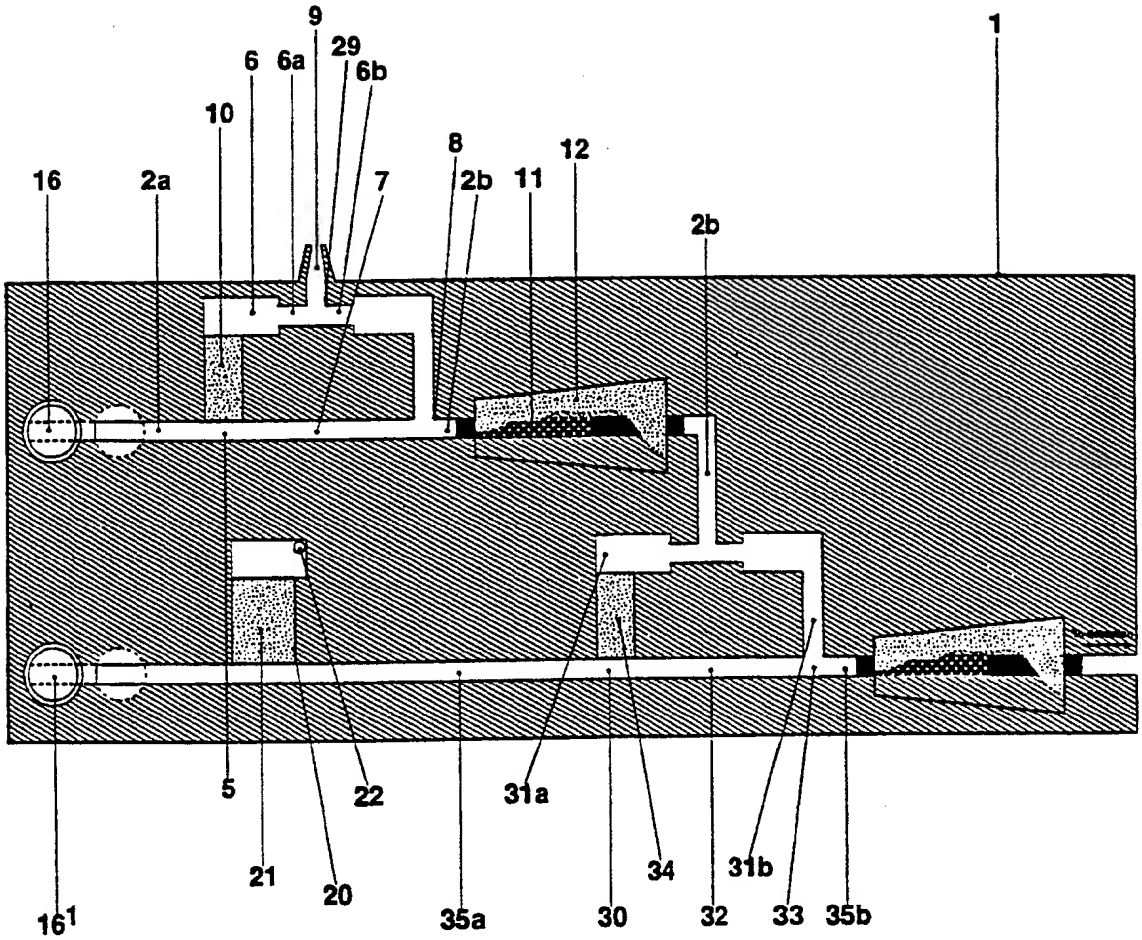

9
29
6
6a
6b
10
8
12
16
2a
7
2b
11
2b
1
5
22
31a
21
20
34
31b
16
1
35a
30
32
33
35b

fig. 6

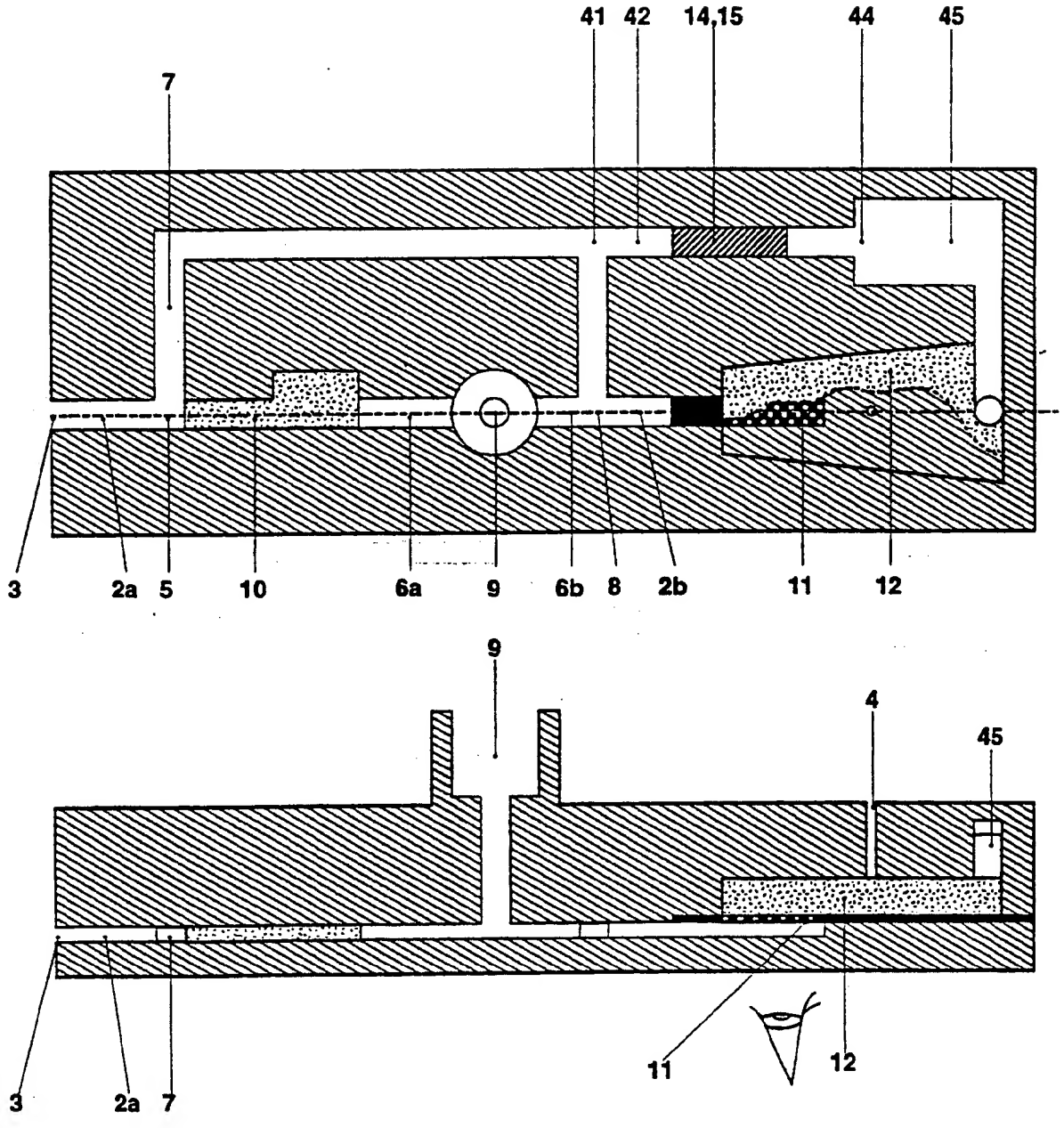


fig. 7

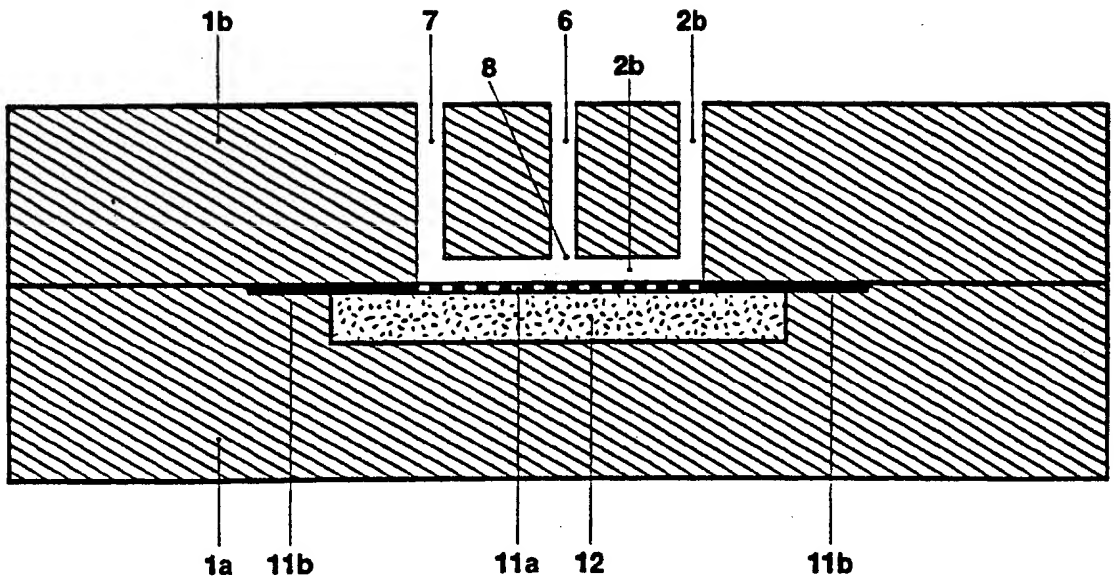


fig. 8

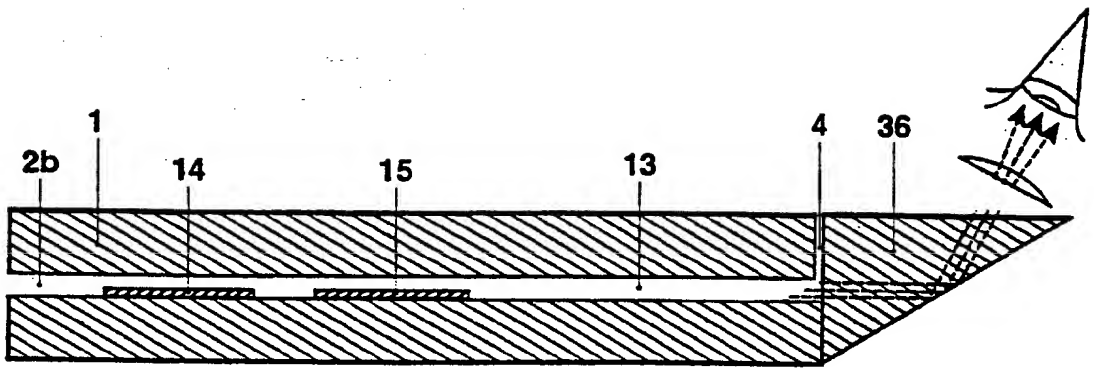


fig. 9

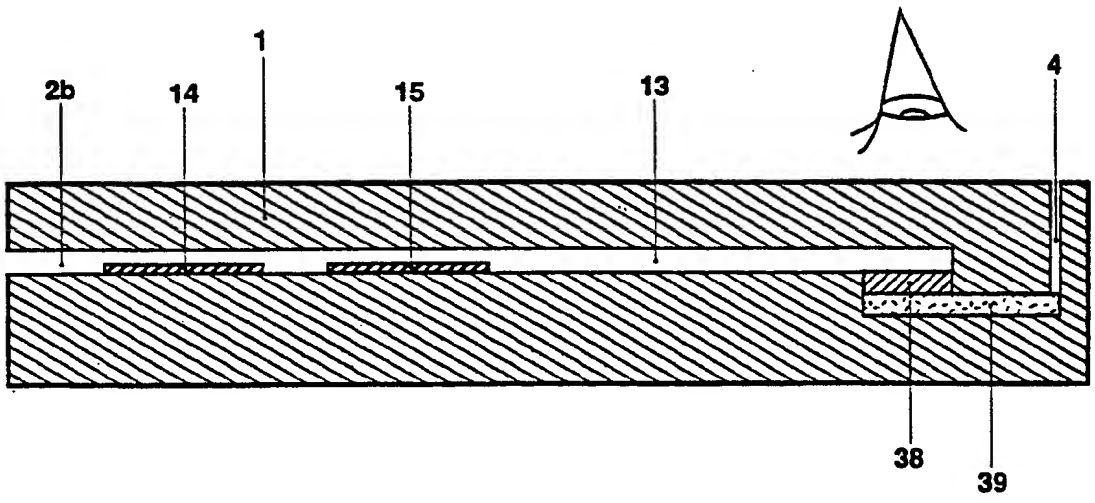
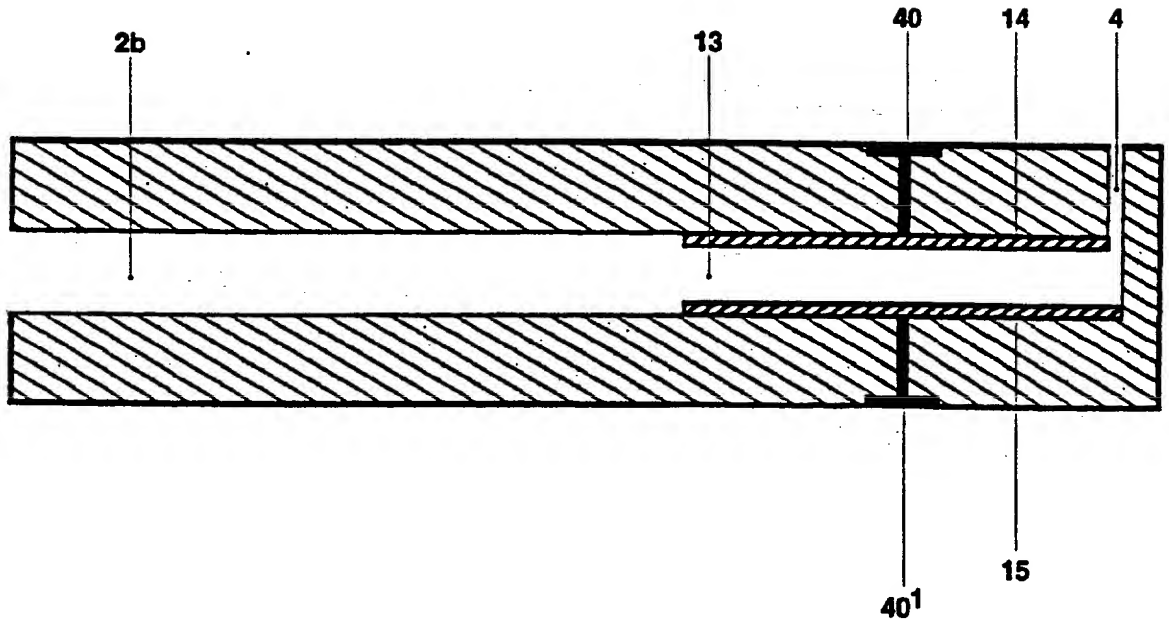


fig. 10



INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 96/03380

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543 G01N33/558 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 590 695 (R.A. BUNCE ET AL.) 6 April 1994	1
A	see column 4, line 10 - column 7, line 6; figures 1-6 see column 15, line 39 - line 43; figure 39	2,3

Y	EP,A,0 281 201 (PB DIAGNOSTIC SYSTEMS INC) 7 September 1988	1
A	see the whole document	2,4-6,9, 10,12,13

A	WO,A,93 10457 (R.A. BUNCE ET AL.) 27 May 1993	1-4,6, 11-14
	see page 2, line 2 - page 5, line 33; figures 1-4	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 1996	09. 12. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	De Kok, A

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 96/03380

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 262 328 (ABBOTT LABORATORIES) 6 April 1988 see the whole document	1-3,6
A	US,A,5 256 372 (P.C. BROOKS ET AL.) 26 October 1993 see the whole document	1,7,8
A	EP,A,0 545 500 (EASTMAN KODAK COMPANY) 9 June 1993 see the whole document	1,4,5
A	EP,A,0 306 336 (SYNTEX INC) 8 March 1989 see column 27, line 15 - column 28, line 50	1-3,9
A	EP,A,0 314 499 (UNILEVER PLC) 3 May 1989 see the whole document	1-4, 11-14

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/EP 96/03380

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0590695	06-04-94	AT-T-	126353	15-08-95
		DE-D-	69021470	14-09-95
		DE-T-	69021470	25-01-96
		EP-A-	0417243	20-03-91
		WO-A-	9011519	04-10-90
		GB-A,B	2231150	07-11-90
		JP-T-	3505261	14-11-91
		US-A-	5198193	30-03-93
		US-A-	5354538	11-10-94
EP-A-0281201	07-09-88	US-A-	4918025	17-04-90
		AU-B-	605257	10-01-91
		AU-A-	1294988	26-09-88
		CA-A-	1300006	05-05-92
		DE-A-	3880531	03-06-93
		FI-B-	96639	15-04-96
		JP-B-	6016047	02-03-94
		JP-T-	1502526	31-08-89
		NO-C-	173410	08-12-93
		WO-A-	8806731	07-09-88
		ZA-A-	8800550	30-08-88
WO-A-9310457	27-05-93	EP-A-	0613559	07-09-94
		GB-A,B	2261283	12-05-93
		JP-T-	7501148	02-02-95
		US-A-	5540888	30-07-96
EP-A-0262328	06-04-88	US-A-	4960691	02-10-90
		AU-B-	598871	05-07-90
		AU-A-	7903187	31-03-88
		CA-A-	1303493	16-06-92
		DE-A-	3783845	11-03-93
		JP-B-	7036017	19-04-95
		JP-A-	63096559	27-04-88
		US-A-	5310650	10-05-94
US-A-5256372	26-10-93	NONE		
EP-A-0545500	09-06-93	CA-A-	2084097	06-06-93

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte ɔnal Application No
PCT/EP 96/03380

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0306336	08-03-89	US-A-	4981786	01-01-91
		CA-A-	1333046	15-11-94
		DE-D-	3887941	31-03-94
		DE-T-	3887941	01-09-94
		ES-T-	2051303	16-06-94
		JP-A-	1072066	16-03-89
EP-A-0314499	03-05-89	AU-B-	629525	08-10-92
		AU-A-	2786989	23-05-89
		DE-A-	3877696	04-03-93
		WO-A-	8903992	05-05-89
		JP-B-	6014037	23-02-94
		JP-T-	2501504	24-05-90
		US-A-	5275785	04-01-94